



University of Groningen

Enzymic synthesis of cyclothiomaltins

Bornaghi, Laurent; Utile, Jean-Pierre; Penninga, Dirk; Schmidt, Andreas K.; Dijkhuizen, Lubbert; Schulz, Georg E.; Driguez, Hugues

Published in:
Chemical Communications

DOI:
[10.1039/cc9960002541](https://doi.org/10.1039/cc9960002541)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1996

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Bornaghi, L., Utile, J.-P., Penninga, D., Schmidt, A. K., Dijkhuizen, L., Schulz, G. E., & Driguez, H. (1996). Enzymic synthesis of cyclothiomaltins. *Chemical Communications*, 41(22), 2541 - 2542.
<https://doi.org/10.1039/cc9960002541>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Enzymic synthesis of cyclotiomaltins

Laurent Bornaghi,^a Jean-Pierre Utille,^a Dirk Penninga,^b Andreas K. Schmidt,^c Lubbert Dijkhuizen,^b Georg E. Schulz^c and Hugues Driguez^{*a†}

^a Centre de Recherches sur les Macromolécules Végétales, ‡ CNRS, BP 53, 58041 Grenoble Cedex 9, France

^b Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Kerklaan 30, 9751 NN, Haren, The Netherlands

^c Institut für Organische Chemie und Biochemie der Universität, Albertstrasse 21, D-79104 Freiburg i. Br., Germany

The effective conversion of 4-thio- α -maltosyl fluoride **1** into cyclotiomaltins **2**, **3** and **4**, using cyclodextrin glycosyltransferase enzymes from *Bacillus circulans*, is described.

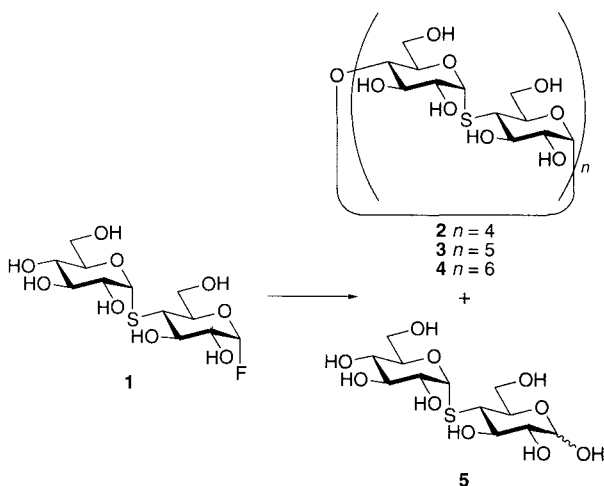
Cyclodextrin glycosyltransferases (CGTase, EC 2.4.1.19) are bacterial enzymes that catalyse the transformation of starch and related maltooligosaccharides to cyclodextrins (CDs), mainly cyclo- α (1 \rightarrow 4)-glucohexaoside (α -CD), cyclo- α (1 \rightarrow 4)-glucoheptaoside (β -CD) and cyclo- α (1 \rightarrow 4)-glucooctaoside (γ -CD).^{1,§} However, cyclo- α (1 \rightarrow 4)-glucononaoside (δ -CD) and cyclo- α (1 \rightarrow 4)-glucododecaoside (η -CD) have also recently been isolated in minute amounts and characterised.² In 1983, enzymic conversion of α -maltosyl fluoride into α -, β - and γ -CDs was demonstrated,³ and since then, this approach has been used for the synthesis of regioselectively substituted CDs starting from α -maltosyl or maltotriosyl fluorides modified at their non-reducing C-6 position.^{4,5} We describe here an efficient synthesis of new cyclo- α (1 \rightarrow 4)-4-thiomaltotetraoside **2**, -thiomaltopentaoside **3** and -thiomaltohexaoside **4** from 4-thiomaltosyl fluoride **1** using CGTase enzymes.

Attempts to obtain cyclotiomaltins using this approach have already been published,⁴ but only linear 4-thiomaltosyl dimer and trimer were formed in low amounts when an impure commercial preparation of CGTase enzyme was used. In order to expand our knowledge of carbohydrate-CGTase protein interactions and to have access to new cyclodextrins for supramolecular studies, we re-investigated the reaction of 4-thio- α -maltosyl fluoride **1** and pure CGTase 1 from *Bacillus circulans* strain 8⁶ and CGTase 2 from *B. circulans* strain 251.⁷ Although comparison of the three-dimensional structures of the two *B. circulans* enzymes reveals that nearly all of the

25% differences are on the surface of the molecules,⁸ we might expect that they present some difference in their specificity.

In the first set of experiments, 4-thio- α -maltosyl fluoride **1** was incubated with CGTase 1 or CGTase 2 and the enzymatic mixtures were analysed by thin layer chromatography (TLC) on silica plates and high performance liquid chromatography (HPLC) using a μ -Bondapak NH₂ column. The TLC patterns and HPLC profiles were identical and showed that oligomerisation occurred. After treatment with β -amylase, an *exo*-glucanase which hydrolyses the penultimate bond of the non-reducing end of linear maltooligosaccharides and which is unable to attack cyclodextrins,⁹ linear hemithiomaltodextrins were converted into 4-thiomaltose **5**. Under the conditions used,[¶] the time-course of the reaction shows that the optimum time for recovery of cyclotiomaltins is around 10 h, and that no interconversion occurred between these cyclic compounds. The enzymatic mixture was treated as described and purified by preparative HPLC using μ -Bondapak NH₂ column. Cyclotiomaltins **2**, **3** and **4** were isolated in 16, 14 and 7% yield respectively. Importantly, it should be noted that with the same quantity of CGTase 2 enzyme, α -maltosyl fluoride afforded a mixture of α -, β - and γ -CDs after only 1 h with β -CD as the predominant product, while maltotriose was hydrolysed into D-glucose and maltose. It is interesting that, in the first experiment, cyclo- α (1 \rightarrow 4)-4-thiomaltotriose was not obtained and only the linear hemithiomaltohexaose was observed when the β -amylase treatment was omitted. The shift in the reaction products towards larger CD ring sizes may be explained by the high flexibility of conformation of the 4-thiomaltosyl residues.^{10,11} The complexation properties of these new molecules and their biochemical properties will be described in due course.

This work was funded by Research Grant B102 CT943008 from the European Community.



Scheme 1

Footnotes

[†] E-mail: hdriguez@cermav.grenet.fr

[‡] Affiliated to Université Joseph Fourier de Grenoble.

[§] We decided to use the nomenclature proposed by Lichtenthaler and Immel for the natural compounds,^{1b} and we propose the generic name of cyclotiomaltins for these new compounds consisting of 4-thiomaltosyl repeating units.

[¶] CGTase 2 (19.5 U cm⁻³, 60 mm³) was added to a solution of compound **1** (55 mg, 0.15 mmol) in phosphate buffer (0.2 mol dm⁻³, pH 6.5, 5 cm³). The mixture was incubated at 40 °C for 10 h. The reaction was stopped by boiling for 10 min and then the proteins eliminated by spinning. The supernatant was freeze-dried, diluted in the same phosphate buffer (1.25 cm³) and then treated with β -amylase (20 U cm⁻³, 1 mm³) at 40 °C for 24 h. After boiling, the reaction mixture was treated with TMD-8 mixed bed resin (Sigma, St Louis, MO USA), freeze-dried and purified on HPLC (μ -Bondapak NH₂ column, Interchim, Montluçon, France) with a 60:40 MeCN–water mixture as eluent. All new compounds gave satisfactory high resolution mass spectra.

References

- 1 (a) D. French, *Adv. Carbohydr. Chem.*, 1957, **12**, 189; (b) F. W. Lichtenthaler and S. Immel, *Tetrahedron: Asymmetry*, 1994, **5**, 2045.
- 2 T. Endo, H. Ueda, S. Kobayashi and T. Nagai, *Carbohydr. Res.*, 1995, **269**, 369 and references cited therein.
- 3 E. J. Hehre, K. Mizokami and S. Kitahata, *Denpun Kagaku*, 1983, **30**, 70 (*Chem. Abstr.*, 1983, **99**, 84236 m).
- 4 S. Cottaz, C. Apparü and H. Driguez, *J. Chem. Soc., Perkin Trans. I*, 1991, 2235.
- 5 C. Apparü, S. Cottaz, C. Bosso and H. Driguez, *Carbohydr. Lett.*, 1995, **1**, 349.
- 6 C. Klein, J. Hollender, H. Bender and G. E. Schulz, *Biochemistry*, 1992, **31**, 8740.
- 7 D. Penninga, B. Strokopytov, H. J. Rozeboom, C. L. Lawson, B. W. Dijkstra, J. Bergsma and L. Dijkhuizen, *Biochemistry*, 1995, **34**, 3368.
- 8 C. L. Lawson, R. van Montfort, B. Strokopytov, H. J. Rozeboom, K. H. Kalk, G. E. de Vries, D. Penninga, L. Dijkhuizen and B. W. Dijkstra, *J. Mol. Biol.*, 1994, **236**, 590.
- 9 J. A. Thoma and D. E. Koshland, *J. Am. Chem. Soc.*, 1960, **82**, 3329.
- 10 K. Mazeau and I. Tvaroska, *Carbohydr. Res.*, 1992, **225**, 27.
- 11 K. Bock, J. O. Düs and S. Refin, *Carbohydr. Res.*, 1994, **253**, 51.

Received, 16th July 1996; Com. 6/04999K